example, in the majority of families with LEOPARD Syndrome-associated HCM, mutations occur in the protein tyrosine phosphatase SHP2. We therefore hypothesized that the sarcomeric mechanical properties are altered by SHP2 mutations. Transgenic mice were generated with cardiac myocyte-specific expression of a loss-of-function mutation of SHP2 (Q510E-SHP2). These mice developed an early-onset form of HCM with increased myocyte size, heart-to-body weight ratios, and interventricular septum thickness. Interestingly, we found that permeabilized cardiac myocyte preparations from 1-month old Q510E-SHP2 mice displayed greater maximal Ca^{2+}-activated tension (SHP2 = 84 ± 2 kN/m^2; Wt = 45 ± 14 kN/m^2) and power generating capacity (SHP2 = 10 ± 2 μW/mg; Wt = 5 ± 1 μW/mg) compared to myocytes from littermate wildtype (Wt) mice. We also found PKA-mediated phosphorylation of both myosin binding protein-C (MyBP-C) and cardiac troponin I (cTnI) was increased in cardiac myofibrils from 1 month old Q510E-SHP2 mice. Taken together, these results implicate a compensatory increase in the contractile state of cardiac myofibrils in response to loss of SHP2 function.

1805-Pos Board B575
DCM-Linked D230N Tropomyosin Mutation Results in Early Dilatation and Systolic Dysfunction in Mice
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Recently, a study in two large multi-generational families described a familial dilated cardiomyopathy (DCM) caused by a single amino acid substitution Asp230Asn (D230N) in tropomyosin. These families demonstrated a unique bimodal disease distribution in which infants presented with a severe form of DCM, while adults presented with a mild to moderate clinical phenotype. To determine the biophysical consequences of this mutation on tropomyosin and its effects on regulatory function in the sarcomere, we employed circular dichroism and the regulated in vitro motility assay. We found that while this mutation does not affect overall thermal stability of tropomyosin, it has a profound effect on regulatory function. As previously shown in solution, the presence of the D230N mutation decreases the maximal velocity of filament sliding and calcium sensitivity of thin filament activation compared to wild type filaments. Additionally, the D230N mutation increases the cooperativity of myofilament activation. In order to further explore our biophysical observations and the physiologic effects of the D230N mutation, we created a transgenic murine model. In mice carrying the D230N tropomyosin mutation we found evidence of early dilatation and systolic dysfunction by echocardiogram in the absence of histological changes such as fibrosis or inflammatory cell invasion. Ultrastructural analysis of transgenic left ventricular tissue demonstrated z-disk alterations. Finally, preliminary studies on isolated myocytes from transgenic mice loaded with fura-2AM demonstrate no discernible differences in calcium transients compared to non-transgenic siblings suggesting that functional impairments are not due to calcium handling defects. Collectively, these studies suggest that the D230N mutation in tropomyosin is responsible for alterations in structure and function of the thin filament that result in a primary dilatation of the cardiac left ventricle. *This work is supported by funding from the Children's Cardiomyopathy Foundation.*

Muscle: Fiber & Molecular Mechanics & Structure II

1806-Pos Board B576
Geometric Changes of Transverse Tubules in Rabbit Cardiac Myocytes during Contraction
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Invaginations of the sarcolemma, called 'transverse tubules' (t-tubules), allow for rapid communication of electrical activation deep into the interior of ventricular cardiomyocytes. Given the length and radius of t-tubules, the rate of diffusion alone appears to be insufficient for homeostasis of the t-tubular content, especially during exercise [1]. Previously, we reported in rabbit ventricular myocytes that positive longitudinal strain modulates geometrical features of t-tubules [2] and their mouth-region [3] in a way that could support convective re-distribution of t-tubular content. Here, we test the hypothesis that cell contraction also affects t-tubular volume.
Isolated ventricular cardiomyocytes from adult New Zealand white (NZW; n=23) rabbits were imaged using an inverted confocal microscope [2] either at slack length or during negative strain to ~85%, caused by exposure to superfusate-induced tonic contracture. Image stacks of cell segments were deconvolved and t-tubules segmented. In addition, ventricular tissue from NZW rabbit hearts, fixed either at zero intra-ventricular pressure (n=2) or during contracture (n=2), was studied using transmission electron microscopy (TEM; [3]). T-tubular length and volume were assessed in confocal images, while ellipticity and orientation were explored both in confocal and TEM data. Statistical significance was determined using a two-tailed t-test with $p<0.05$ considered significant.
T-tubular cross-section changed, reducing ellipticity in contractured myocytes (compared to control), while apparent length and total volume of t-tubules increased. This suggests that both passive distension and active contraction may give rise to a convective component of fluid transport, mixing, and exchange of t-tubular content.
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1807-Pos Board B577
Mechanoregulation of Delayed Stretch Activation
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Delayed stretch activation (SA) is a prominent feature in the function of the cardiac myocyte and plays an important role in regulating cardiac output. A mechanistic understanding of SA is essential for the development of models that quantitatively and causally connect molecular defects to global cardiac function. We propose a novel mechanism that defines how mechanical forces imposed by stretch affect troponin-actin and myosin-actin bonds and thereby modify calcium-modulated thin filament regulation. Tropomyosin molecules are assumed to form two continuous flexible chains (CFC) along each actin filament; tropomyosin movements are restricted by bound troponins and myosin heads bound to actin. Crossbridges transmit sarcomere forces to the thin and the thick filaments. A stretch applied on a sarcomere extends the thin filaments and associated CFCs imposing additional strain (via the CFC) on the TnI-actin and myosin-actin bonds. The spatial positions of these bonds were calculated using the computational platform, MUSICO (**MU**scle **SI**mulation **CO**de) and, hence, the forces acting on TnI-actin and myosin-actin bonds before and after stretch at different Ca^{2+} concentrations. These forces were assessed from finite element analysis of CFCs weakly interacting with the actin surface and strongly interacting with actin via Tn attachments to actin. An imposed stretch leading to sarcomere forces of ~50% of the maximum isometric force increased the forces on the bonds by more than 10 pN, sufficient to strongly tilt the energy landscapes and accelerate the rate of detachment of Tn from actin, even without Ca^{2+} bound to TnC. The maximum effect of this behavior is observed in muscle fibers at submaximal activation (pCa ~ 6). This analysis suggests a mechanism for observed modulation of cardiac myocyte contractility by SA based on altered mechanochemistry of thin filaments regulation via CFC. Supported by NIH R01 AR048776 and R01 DC 011528.

1808-Pos Board B578
A Model for Ca(2+)-Dependent Cooperative Activation in the Cardiac Thin Filament that Allows for Crossbridge Cycle Feedback
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The mechanism underlying the apparent cooperativity of the cardiac thin filament was investigated by using FRET to follow the N-domain opening of recombinant cardiac troponin C (N-cTnC opening) passively exchanged into rat myocardial fiber bundles and tested under a variety of experimental conditions. Calcium titrations conducted in the presence of crossbridge cycle modulators and tension recovery experiments revealed: 1) N-cTnC opening occurred just as ''cooperatively'' with or without crossbridge cycle activity; 2) the calcium sensitivity of N-cTnC opening is enhanced by crossbridge cycling; 3) the rigor state results in no cooperativity and 10% of the cTnC ensemble being apparently open under resting conditions; 4) the breaking of crossbridges preceding a tension recovery phase results in a slight relaxation of the FRET distance associated with N-cTnC opening, which recovers during tension recovery. In light of this and other evidence from the literature, a model of apparent cooperativity based on the three state model is proposed wherein the steep force-Ca^{2+} relationship ultimately arises from a tunable cTnC binding affinity for Ca^{2+}. Activation may be mathematically modeled by a sum of weighted Hill-equation fractions (i.e. $[L]^n / (K_d + [L]^n)$) with each fraction governed by a hill coefficient of n=1 and representing a particular cTnC conformation with a unique affinity for Ca^{2+}, or ''affinity-state''. The affinity-state of an individual cTnC is influenced by the activation state of its neighboring

regulatory units. Thus the apparent cooperativity of activation is an emergent property caused by the ensemble of cTnC populating distinct affinity-states that arise from the coordinated super architecture of the sarcomeric lattice; due to the nature of the cTnC-Ca^{2+} binding event, cTnC is biased toward binding Ca^{2+} in affinity-states with higher Ca^{2+}-affinity as activation progresses.

1809-Pos Board B579

Incorporating Cooperativity into Huxley-Type Cross-Bridge Models in Thermodynamically Consistent Way

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We present a mathematical model of actomyosin interaction, as a further development of a cross-bridge model that links mechanical contraction with energetics [Vendelin et al., Ann. Biomed. Eng.: 28: 629, 2000]. The former model is composed of the Huxley-type model for cross-bridge interaction and the phenomenological model of calcium - induced activation. The purpose of the new model was to replace the phenomenological description. To introduce mechanistic description of the activation, cooperativity effects should be taken into account.

The aim of this work is to incorporate cooperativity into Huxley-type cross-bridge model in thermodynamically consistent way.

While the Huxley-type models assume that cross-bridges act independently from each other. Here we take into account that each cross-bridge is influenced by its neighbors. We assume that the muscle contraction can be described by ensemble of cross-bridge groups. For simplicity, the groups consist of five consequative cross-bridges, out of which the first and the last ones are always in unbound state as boundary conditions. Cooperativity is introduced by taking into account that binding of calcium or cross-bridge leads to displacement of tropomyosin. Since tropomyosin connects all cross-bridges in a group, the elastic deformation of tropomyosin will influence free energy of the group as well as reaction kinetics.

The model parameters were found by optimization from the linear relation between oxygen consumption and stress-strain area [Hisano et al., Circ. Res.: 61: 318, 1987] as well as experimentally measured stress dynamics of rat trabecula [Jansse et al., Am. J. Physiol.: 269: H676, 1995]. We have found a good agreement between the optimized model solution and experimental data. In addition, model solutions demonstrate the cooperativity effects.

1810-Pos Board B580

From Single Molecule Fluctuation to Muscle Contraction: A Brownian Model of A.F. Huxley's Hypotheses

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Force generation during muscle contraction is the result of thermally fluctuating, cyclical interactions between myosin and actin, which together form the actomyosin complex.

Normally, these fluctuations are modelled using transition rate functions that are based on muscle fiber behaviour. However, this reduces the predictive power of such models.

Therefore, we propose an alternative approach that incorporates diffusion and uses the direct observations of actomyosin dynamics reported in the literature. We precisely estimate the actomyosin potential bias to obtain a Brownian ratchet model that reproduces the complete cross-bridge cycle.

The model is validated by simulating several macroscopic experimental conditions, while its interpretation is compatible with two different force-generating scenarios.

1811-Pos Board B581

Reverse Computational Modeling: From Muscle Mechanics to the Function of Sarcomeric Proteins

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Spatially-explicit mathematical models of muscle sarcomeres (e.g. Daniel et al., 1998, Smith et al., 2008) incorporate information about the position of each molecule in the myofilament lattice and are useful because they can reproduce geometrical effects due to filament architecture and the variable alignment of actin binding sites and myosin heads. Their main disadvantage is that they have many more parameters (for example, filament stiffness values, Ca^{2+} binding affinities, etc.) than conventional Huxley-type models. As a result, most spatially-explicit models to date have been used to make 'forward predictions'. That is, investigators have assigned a plausible value to each model parameter and then run simulations to predict contractile function (tension-pCa curves, measurements of tension recovery, etc.) under various conditions. This can determine the impact on muscle function of altering the biophysical properties of a selected sarcomeric protein. We have developed our own spatially-explicit model (FiberSim) and are currently using it in the alternative 'reverse' direction. Our aim is to predict the functional behavior of many sarcomeric proteins by adjusting the values of the parameters that describe their biophysical behaviors until the resulting simulations match real experimental data records. We have recently succeeded in reproducing tension-recovery (k_{tr}) records obtained at different levels of Ca^{2+} activation using permeabilized myocardial samples from diabetic rats. These samples only contain the slow β isoform of myosin heavy chain and the simulations thus predict a complete kinetic scheme for this isoform's interactions with actin. We are currently extending our work to predict the fast α isoform's kinetic scheme as well by optimizing the fit between the simulations and k_{tr} records obtained from control myocardial samples which contain 30% α and 70% β myosin.

1812-Pos Board B582

Cardiomyopathy-Related Mutations (E244D, K247R, D270N, and K273E) in the H2-Helix of Cardiac Troponin T Have Varied Effects on Myofilament Responsiveness to Calcium and Crossbridge Recruitment Dynamics

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Two hypertrophic cardiomyopathy (HCM)-related mutations, E244D and K247R, and two dilated cardiomyopathy (DCM)-related mutations, D270N and K273E, have been identified along a centralized helix of cardiac troponin T (cTnT). This helix, termed H2(T), is centrally located in the core domain of cardiac troponin, interacting with cardiac troponin I and troponin C. This indicates a functional role of H2(T) in translating conformational changes sensed in troponin C and troponin I to the rest of the thin filament, but this structure-function relationship is not well understood. To determine the significance that disease-related alterations in the structure of H2(T) have in altering contractile function, we measured the contractile properties of rat cardiac muscle fibers containing rat cTnT variants $cTnT_{E245D}$, $cTnT_{K248R}$, $cTnT_{D271N}$, or $cTnT_{K274E}$ corresponding to human E244D, K247R, D270N, or K273E mutations, respectively. We measured simultaneous force production and ATPase activity, as well as force responses ($F(t)$) to step-length perturbations in demembranated cardiac muscle fibers activated at various [Ca^{2+}], at both sarcomere lengths 2.0 and 2.3 μm. Fibers containing $cTnT_{K274E}$ exhibited an increase in myofilament Ca^{2+} sensitivity when compared to those containing wild-type (WT)-cTnT. In addition, crossbridge recruitment dynamics, as estimated by model-predictions of $F(t)$ and k_{tr} measurements, were slower in fibers containing $cTnT_{K274E}$, a trend that was also seen in fibers containing $cTnT_{D271N}$. Ca^{2+} sensitivity of fibers containing $cTnT_{K248R}$ or $cTnT_{D271N}$ was less than that of fibers containing WT-cTnT. Furthermore, maximal ATPase activity was slightly but significantly increased in fibers containing $cTnT_{E245D}$ or $cTnT_{K248R}$. These findings suggest that mutations along H2(T) influence the cTnT-modulated mechanisms of myofilament activation and crossbridge recruitment dynamics, and may contribute in part to cardiac dysfunction associated with HCM and DCM diseases.

1813-Pos Board B583

Changes in the Myocardial Expression of Tropomyosin Isoforms Modulate Troponin T-Mediated Cardiac Thin Filament Activation

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The spatial distribution of the troponin complex (Tn) on the thin filament, and as a result, the functional role of Tn in cardiac thin filament activation depends on structural interactions between the N-terminus of troponin T (T1) and the overlapping ends of tropomyosin (Tm). Thus, T1-Tm interactions have an influential role in regulating cardiac thin filament activation. Structural alterations in T1 have been shown to diminish cardiac activation and in view of the physical interactions between T1 and Tm, it is conceivable that the effect of T1 on cardiac activation can also be modulated by structural alterations in Tm. However, there is a lack of understanding of how structural changes in Tm influence T1-mediation of cardiac activation. To better understand how structural changes in Tm influence T1-mediated cardiac activation, we studied contractile function by reconstituting mouse cardiac troponin T (McTnT) deletion proteins, McTnT 1-44 deletion and McTnT 45-74 deletion, onto detergent-skinned papillary fibers isolated from hearts of transgenic mice expressing β-Tm. Control experiments were performed by reconstituting the McTnT 1-44 deletion and McTnT 45-74 deletion proteins onto detergent-skinned papillary fibers isolated from hearts of wild-type mice containing α-Tm. Our preliminary results show that the T1 deletions induced significant functional alterations in wild-type fibers. Interestingly, the T1-deletion-induced alteration of cardiac function was further modulated by the myocardial expression of β-Tm. For example, the McTnT 1-44 deletion-induced reduction